At the outset, the Examiner is respectfully requested to the comments of Mr. Kazunori Saitoh, an inventor in the present application, submitted herewith. Mr. Saitoh explains in detail the reasons why the claimed invention is so advantageous that the same level of performance is not provided by other classical techniques. Mr. Saitoh also explains the significance of the recitation that the one of the antibodies "does not have strict specificity for the antigen" in the claimed method.

In addition, the rejections of the claims under 35 U.S.C. §102(b) or, in the alternative, under 35 U.S.C. §103(a) over Strahilevitz (U.S. Patent No. 4,375,414) or EP 617 285 (EP '285) are respectfully traversed. These references fail to disclose or suggest the claimed immunoassay method.

Strahilevitz discloses immunological methods for removing species from the blood (see the Abstract). The method described in the reference involves the use of two antibodies (see the Figures). However, Strahilevitz fails to disclose using one antibody having high specificity for the antigen while the other antibody does not have strict specificity for the antigen. Accordingly, this reference fails to disclose the claimed methods.

EP '285 discloses a method for reducing the Hook Effect in immunoassays with particulate carriers (see the Abstract). The method described in this reference involves the use of two different antibodies (see pages 3-4). However, EP '285 fails to describe using one antibody having high specificity for the antigen while the other antibody does not have strict specificity for the antigen. Accordingly, this reference fails to disclose the claimed methods.

Moreover, neither Strahilevitz nor EP '285 suggest the claimed methods. Both Strahilevitz and EP '285 fail to suggest an immunoassay in which one of the antibodies has high specificity for the antigen while the other antibody does not have strict specificity for the antigen. Accordingly, neither reference suggests the claimed methods.

Strahilevitz and EP '285 fail to disclose or suggest the claimed methods. Accordingly, Claims 7-34 are not anticipated by or obvious over these references. Withdrawal of these grounds of rejection is respectfully requested.

Moreover, the rejection of the claims under 35 U.S.C.§103(a) over Cragle et al in view of Strahilevitz and EP '285 is respectfully traversed. These references fail to suggest the claimed immunoassay method.

As recognized by the Examiner, Cragle et al fails to disclose sequential contact of two antibodies (see the Official Action dated July 12, 1999, at page 7, first full paragraph). In addition, Cragle et al fails to disclose that one of the antibodies has high specificity for the antigen while the other antibody does not have strict specificity for the antigen.

Both Strahilevitz and EP '285 fail to suggest an immunoassay in which one of the antibodies has high specificity for the antigen while the other antibody does not have strict specificity for the antigen, as discussed above.

Accordingly, the combined teachings of the cited references fail to suggest the claimed methods. Claims 7-34 are not obvious over Cragle et al in view of Strahilevitz and EP '285. Withdrawal of this ground of rejection is respectfully requested.

The rejection of the claims under 35 U.S.C. §112, second paragraph, is respectfully traversed. In Claims 7 and 21 measuring the amount of agglutinate as recited in (b) of each claim accomplishes the goal set forth in the preamble of these claims. In view of Mr. Saitoh's comments, the meaning of the recitation that one of the antibodies "does not have strict specificity for the antigen" would be readily understood by one skilled in the art. Accordingly, the claims are definite within the meaning of 35 U.S.C. §112, second paragraph. Withdrawal of this ground of rejection is respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618

James J. Kelly, Ph.D.
Registration No. 41,504

22850
Tel. (703) 413-3000
Fax. (703) 413-2220
(OSMMN 11/98)

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Reasons of why the phrase "does not have strict specificity for the antigen" is needed for characterizing the immunoassay of the present invention.

I. Detection specificity in the presence of impurity antibody:

In general, there are two types of antibodies that are roughly divided into monoclonal antibody and polyclonal antibody. Polyclonal antibody can be obtained as a serum containing immunoglobulins which are generated through the immune response triggered by injecting into a host animal with a targeted antigen (i.e., 'protein' in the present invention). All different extracellular substances can be involved in the immune formation of polyclonal antibody as far as they are recognized as non-self by the immunoreaction system of the host animal. Therefore, if the targeted antigen has been contaminated by the other pathogens, the polyclonal antibody tends to become an impurity polyclonal antibody which faithfully reflects on the various natures of all different extracellular substances involved. In the formation process of a polyclonal antibody, it is essential to succeed in purification of the targeted antigen from such contamination. This process nevertheless requires highly sophiscated techniques and is thought to be costly disadvantageous.

When an immunoassay method (i.e., 'nephelometry' in the present invention) is carried out containing such an impurity antibody as one of its materials, it is almost inevitable to allow two different complexes to form in blood, one of which is an immune complex consisting of the targeted antigen and its corresponding specific antibody, and the other of which is a immune complex consisting of an impure mixture of various antigens and the non-specific impurity antibodies. In the reaction process of nephelometry, the immune complexes grow up into massive agglutinates by use of polyethylene glycol (reaction accelerator), and an optical change (change in absorbance) is induced as a result thereof. But this process does not contribute to the framework for recognizing the difference of the above-mentioned specific complex from the above-mentioned non-specific

complex. Therefore, any classical methods have been required to calculate the sum of both absorption changes, thereby determine a differentiation.

Compared with such classical methods, the present invention's immunoassay is capable of avoiding the occurrence of a non-specific immunoreaction associated with the impurity antibody, because this method has a unique technique characterized by immobilizing a monoclonal antibody specific to the targeted antibody on an insoluble carrier and also using a polyclonal antibody as a free antibody. Among the immune complexes that could be formed in the present immunoreaction, there are the following three complexes, i.e., (1) a complex consisting of the targeted antigen and its specific immobilized monoclonal antibody; (2) a complex consisting of the targeted antigen and its specific antibody in the free antibody (in the case where the specific antibody and the impurity antibody coexist),; and (3) a complex consisting of the various antigens contaminated and the corresponding impurity antibodies. In essence, the present invention's immunoassay system requires no use of polyethylene glycol, and therefore, these immune complexes are transiently kept inactive to more grow into their optically detectable shape. Following this first phase reaction, a further complex is generated as a second phase complex coupled with both complexes (1) and (2) mentioned above. This second phase complex continues its growth until its optical change takes place in accordance with the principle of the nephelometry method using immobilization. At this phase, the complex (3) plays no influential role on the results of immune detection, because the condition needed for growth is inappropriate to this complex.

For the reasons explained above, it would be quite reasonable to think that the polyclonal antibody of the present invention has a characteristic expressed as "does not have strict specificity for the antigen". Namely, it has become possible by the present invention's immunoassay to achieve good performance for specific detection, even when the targeted antigen is polluted by the other various substances. Thus, the present invention provides greatly simpler and less expensive immunoassay method for preparation of polyclonal antibody than any classical methods.

II. Detection specificity compared between modified antigen and non-modified antigen:

Macromolecules, most typically such as proteins, are well known to have multiple antigenic determinants (epitopes) contributing to development of the corresponding antibodies in a host immune system, which are therefore sometimes called "multivalent antigen". When a maclomolecule is injected into a host animal, multiple antibodies are generated correspondingly against the respect epitopes on its surface through the immunoreaction pathway, not against the macromolecule antigen itself. Polyclonal antibody is an antibody composed of such multiple antibodies. There would be no problem if the targeted antigen were to exist as the only substance in a sample without any contamination by other antigens. But in the case where the targeted antigen emerges as a metabolite of the other substance, a serious problem is caused because the targeted antigen could be closely resemblant with its original substance before metabolized. For example, HbA1c, known as one of glycohemoglobins, is no different from its original counterpart except that its amino acid (valin) has been changed by saccharogenic modification at the terminal site of Hb β chain. Although one can try to make a polyclonal antibody by use of highly purified HbA1c, it is not always possible to completely avoid inclusion of both modified and non-modified types. This indicates that if there are other sites serving as antigenic determinants than such a modified site, it could lead to formation of impure immune complexes with the polyclonal antibody's failure of recognizing the differences of both modified and non-modified types. This type of polyclonal antibody, too, is classified into impurity polyclonal antibody. The "modification" is usually performed by methods such as saccharification, oxidation and calbamylation, but these methods are too limited to bring significant differences into determination of both modified and non-modified types. So far, it has been extremely difficult to achieve good performance with excellent detection specificity as needed.

For simplicity, the abbreviation "(a)" will be hereinafter used instead of the term "the antigenic determinant of a non-modified antigen", as well as "(a')" for "the antigenic determinant of a non-modified antigen, "(b)" for "the overlapping antigenic determinant of a non-modified antigen", and "(c) for

"the overlapping antigenic determinant of a modified antigen". A sample was prepared to become a condition under which both modified and non-modified substances coexist. Among the complexes formed in the present invention, there are the following three complexes; a complex (I) consisting of an immobilized antibody specific to (a') and the targeted antigen (modified protein), a complex (II) consisting of free antibodies (against (b) and (c)) and the modified protein, and a complex (III) consisting of free antibodies (against (b) and (c)) and non-modified protein. Complex (I) and complex (II) interact with each other and develop into a voluminous complex, resulting in formation of their agglutinate having the size enough to induce optical changes. On the other hand, complex (III) is impossible to develop into such optically detectable size, because the present condition requires no use of polyethylene glycol as an accelerator of the development. This indicates that the complex (III) plays no influential role on the accuracy of the immune detection.

As discussed above, we are of the opinion that since the immunoassay of the present invention is well functional even when the extremely resemblant antigens are used for preparation of the polyclonal antibody, there is no reason to cast doubt on reasonability of the expression "does not have strict specificity for the antigen" used for characterizing the polyclonal antibody of the present invention. Namely, it has become available to detect even presence of very slight differences between the modified or non-modified, thus leading to development of simpler and less expensive immunoassay method than any classical immunoassay methods.

Measure distinguishable points of the present immunoassay from the hapten type immunoassay.

I. Detection target: The targeted antigens of the present invention are those having at least three antigenic determinants on their surface, especially such as proteins.

II. Detection system: Immobilized phase is one of essential factors in the immonosaay of the present invention. Free antibody is also similarly important as an essential part of this assay. Optical change is not caused in the presence of immobilized antibody alone, but it is

possible when there is a condition that has been set to conjugate three elements: immobilized antibody, antigen and free antibody. The immune reaction system requires no use of immunoreaction accelerators, such as polyethylene glycol.

Dated this 11th day of January, 2001

Kazunori Saitoh

Kazunori SAITOH